Immunohistochemical Evidence for an Increased Oxidative Stress and Carbonyl Modification of Proteins in Diabetic Glomerular Lesions

DAISUKE SUZUKI,* TOSHIO MIYATA,* NOBORU SAOTOME,*
KATSUNORI HORIE,* REIKO INAGI,* YOSHINARI YASUDA,* KOJI UCHIDA,[†]
YUKO IZUHARA,* MITSUNORI YAGAME,* HIDETO SAKAI,* and
KIYOSHI KUROKAWA*

*Molecular and Cellular Nephrology, Institute of Medical Sciences and Department of Internal Medicine, Tokai University School of Medicine, Isehara, Japan; and †Laboratory of Food and Biodynamics, Nagoya University School of Agricultural Sciences, Nagoya, Japan.

Abstract. Advanced glycation end products (AGE) include a variety of protein adducts whose accumulation has been implicated in tissue damage associated with diabetic nephropathy (DN). It was recently demonstrated that among AGE, glycoxidation products, whose formation is closely linked to oxidation, such as carboxymethyllysine (CML) and pentosidine, accumulate in expanded mesangial matrix and nodular lesions in DN, in colocalization with malondialdehyde-lysine (MDA-lysine), a lipoxidation product, whereas pyrraline, another AGE structure whose deposition is rather independent from oxidative stress, was not found within diabetic glomeruli. Because CML, pentosidine, and MDA-lysine are all formed under oxidative stress by carbonyl amine chemistry between protein amino group and carbonyl compounds, their colocalization suggests a local oxidative stress and increased protein carbonyl modification in diabetic glomerular lesions. To address this hypothesis, human renal tissues from patients with DN or IgA nephropathy were examined with specific antibodies to characterize most, if not all, carbonyl modifications of proteins by autoxidation products of carbohydrates, lipids, and amino acids: CML (derived from carbohydrates, lipids, and amino acid), pentosidine (derived from carbohydrates), MDA-lysine (derived from lipids), 4-hydroxynonenal-protein adduct (derived from lipids), and acrolein-protein adduct (derived from lipids and amino acid). All of the protein adducts were identified in expanded mesangial matrix and nodular lesions in DN. In IgA nephropathy, another primary glomerular disease leading to end-stage renal failure, despite positive staining for MDA-lysine and 4-hydroxynonenal-protein adduct in the expanded mesangial area, CML, pentosidine, and acrolein-protein adduct immunoreactivities were only faint in glomeruli. These data suggest a broad derangement in nonenzymatic biochemistry in diabetic glomerular lesions, and implicate an increased local oxidative stress and carbonyl modification of proteins in diabetic glomerular tissue damage ("carbonyl stress").

Diabetic nephropathy (DN) has become one of the main causes of end-stage renal disease. Although several lines of evidence have suggested that poor glycemic control undoubtedly plays a significant role (1,2), the metabolic events responsible for its development are not well understood. Possible mediators of untoward effects of hyperglycemia include advanced glycation end products (AGE) generated by the Maillard reaction through nonenzymatic glycation of protein amino groups (3).

AGE accumulate in plasma and tissue proteins of diabetic patients (4,5), and their accumulation is correlated with the severity of diabetic complications (6,7). Several lines of evidence suggest that AGE may be involved in the development of glomerular lesions of diabetes (8–10). AGE modification indeed alters the structure and function of matrix tissue proteins (3) and, more interestingly, AGE-modified proteins stimulate a variety of cellular responses (11–13) via a specific cell surface receptor (14,15) on several cell types including glomerular mesangial cells (16–18).

AGE comprise a variety of molecular structures, such as N^ε-(carboxymethyl)lysine (CML) (19), pentosidine (20), and pyrraline (21), characterized by different formation mechanisms. We recently demonstrated that glycoxidation products (22), a subclass of AGE that requires both glycation and oxidation for their formation such as CML and pentosidine, accumulate in expanded mesangial matrix and nodular lesions in DN, in colocalization with malondialdehyde (MDA)-lysine, a lipoxidation product (10). However, pyrraline, another AGE structure whose deposition is rather independent from oxidative stress, was not found within diabetic glomeruli (10).

Under oxidative stress, proteins may be modified either directly by reactive oxygen species with the eventual formation of oxidized amino acids or indirectly by reactive carbonyl compounds formed by autoxidation of carbohydrates, lipids, or amino acids. Both glycoxidation products and MDA-lysine are indeed products formed by carbonyl amine chemistry between protein amino group and carbonyl compounds derived from autoxidation of carbohydrates and lipids (23–25). Autoxidation of carbohydrates yields reactive carbonyl compounds, precursors of glycoxidation products, such as glyoxal (23), methylglyoxal (26), and glycolaldehyde (24), as well as dehydroascorbate formed on oxidation of ascorbate (27,28). Lipid peroxidation of polyunsaturated fatty acids yields other reactive carbonyl compounds. Some are identical to those formed from carbohydrates (29), such as glyoxal and methylglyoxal, and others are characteristic of lipids, such as MDA, 4-hydroxynonenal (HNE), and acrolein (30,31). The latter reactive carbonyl compounds produce lipoxidation products, MDA-lysine, HNE-protein adduct, and acrolein-protein adduct, also termed advanced lipoxidation end products. The hydroxyamino acids L-serine and L-threonine are also oxidized and converted to reactive carbonyl compounds, such as glycolaldehyde and acrolein, which are highly reactive with proteins, leading to formation of CML and acrolein-protein adducts (32), respectively.

We therefore hypothesized that the localizations of glycoxidation products and MDA-lysine, both of which are closely related to oxidative process, are independent evidence for a local oxidative stress and increased carbonyl modification of proteins in diabetic glomerular lesions. To address this hypothesis, human renal tissues from patients with DN or IgA nephropathy (IgA-N), another primary glomerular disease leading to end-stage renal failure, were examined with specific antibodies to characterize carbonyl modification of proteins by autoxidation products of carbohydrates, lipids, and amino acids: CML (derived from carbohydrates, lipids, and amino acid), pentosidine (derived from carbohydrates), MDA-lysine (derived from lipids), HNE-protein adduct (derived from lipids), and acrolein-protein adduct (derived from lipids and amino acid). Here, we provide evidence for an increased carbonyl modification of proteins by autoxidation products of carbohydrates, lipids, and amino acids in diabetic glomerular tissue damage, implicating a broad derangement in nonenzymatic biochemistry in diabetic glomerular lesions.

Materials and Methods
Patients

Open renal biopsy specimens were obtained from 15 non-insulin-dependent diabetes mellitus patients with DN and 15 patients with IgA-N. Characteristics of the study population are summarized in Table 1. The presence of DN or IgA-N was confirmed by histologic evaluation of renal biopsy specimens, such as light microscopy, electron microscopy, and immunofluorescence staining. No patients received steroids or immunosuppressive drugs before renal biopsy. Control samples were also obtained from five subjects using uninvolved portions of surgically removed kidneys afflicted with malignancies. These subjects had no urinary abnormalities, and histologic examination of control tissues excluded any glomerular diseases. The study was approved by the Human Research Committee of Tokai University School of Medicine, and informed consent to open renal biopsy and immunohistochemical studies was obtained from each patient.

On the basis of light microscopy findings, diabetic patients were divided into three grades (33), as shown in Figure 1A: grade I (six patients) had mild mesangial expansion, grade II (six patients) had moderate mesangial expansion, and grade III (three patients) had severe mesangial expansion with Kimmelstiel-Wilson nodules. The clinical data of each group of DN are summarized in Table 2. Patients with IgA-N were also divided into three grades (Figure 1B) according to the modified definition of the clinical guideline of IgA nephropathy from the Ministry of Health and Welfare of Japan and the Japanese Society of Nephrology (34): grade I (five patients) had slightly mesangial cell proliferation and mesangial matrix expansion, grade II (five patients) had diffuse mesangial cell proliferation and matrix expansion, and grade III (five patients) had severe, diffuse mesangial cell proliferation and marked matrix expansion. The clinical data of each group of IgA-N are summarized in Table 3.

Antibodies

The antibodies used in the present immunohistochemistry are summarized in Table 4. They include anti-pentosidine rabbit IgG (10,35), anti-HNE-protein adduct rabbit IgG (36), anti-MDA-lysine mouse monoclonal IgG (a gift from Dr. Joseph L. Witztum, University of California-San Diego) (37,38), anti-acrolein-protein adduct mouse IgG (31), and monoclonal anti-AGE mouse IgG (39,40), the major epitope-structure of which was recently identified as CML (28,41). These antibodies recognize distinct structures and do not cross-react with the other structures, as demonstrated by immunoblot analysis (10).

Table 1. Clinical parameters of patients and healthy subjects[a]

Group	Age (yr)	Serum Creatinine (mg/dl)	Total Protein (g/dl)	Fasting Plasma Glucose (mg/dl)	Urinary Protein (g/d)	Creatinine Clearance (ml/min)
DN ($n = 15$)	44.2 ± 10.9	1.06 ± 0.41	6.10 ± 0.74	155.3 ± 41.2[b]	2.18 ± 2.59[c]	82.5 ± 32.9
IgA-N ($n = 15$)	45.8 ± 7.6	1.49 ± 0.91	6.58 ± 0.68	86.0 ± 6.0	1.15 ± 0.76	65.4 ± 17.3[c]
Healthy subjects ($n = 5$)	48.2 ± 2.5	0.82 ± 0.21	6.82 ± 0.58	90.6 ± 10.3	0.04 ± 0.01	95.2 ± 4.6

[a] Data are expressed as mean $\pm$ SD. DN, diabetic nephropathy; IgA-N, IgA nephropathy.
[b] $P < 0.005$ versus healthy subjects.
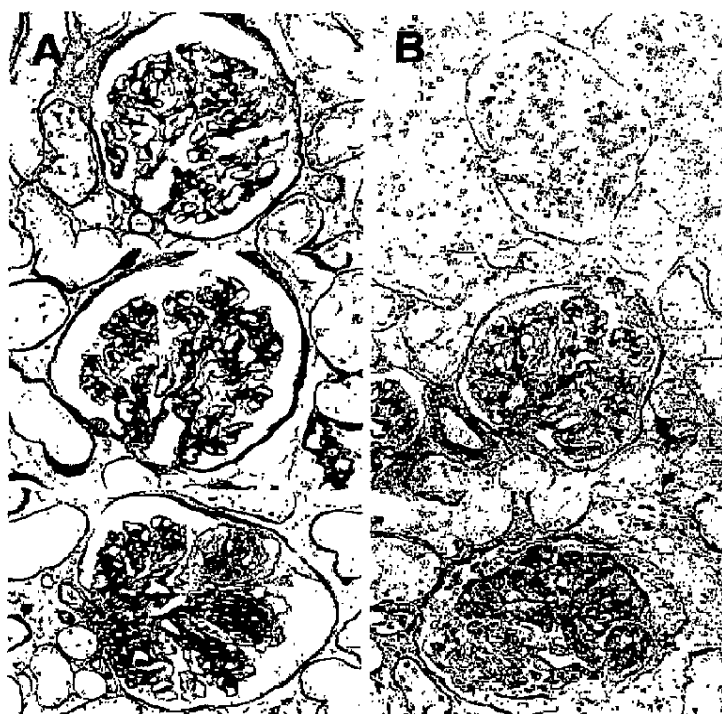[c] $P < 0.05$ versus healthy subjects.

Figure 1. Representative light microscopic pictures of glomeruli from patients with diabetic nephropathy (DN) (A) or IgA nephropathy (IgA-N) (B). Tissue sections from patients with DN were stained by Periodic acid-Schiff and divided into three grades according to the degree of the mesangial matrix expansion (33): grade I (top panel in A), mild; grade II (middle), moderate; grade III (bottom), severe with Kimmelstiel-Wilson nodules. IgA-N tissue sections were divided into three grades according to the degree of the mesangial cell proliferation and mesangial matrix expansion (34): grade I (top panel in B), slight; grade II (middle), diffuse; grade III (bottom), severe. Magnification, ×200.

Table 2. Clinical parameters of patients with DN[a]

Histologic Grade	Age (yr)	Known Duration of Diabetes (yr)	Serum Creatinine (mg/dl)	Total Protein (g/dl)	Fasting Plasma Glucose (mg/dl)	HbA1c (%)	Urinary Protein (g/d)	Creatinine Clearance (ml/min)
DN I ($n = 6$)	38.5 ± 7.6	10.3 ± 5.7	0.73 ± 0.12	6.62 ± 0.56	152.8 ± 37.1	8.97 ± 1.75	0.27 ± 0.25	93.8 ± 25.5
DN II ($n = 6$)	47.5 ± 13.5	8.0 ± 5.9	1.05 ± 0.11[b]	5.92 ± 0.59	167.2 ± 51.5	9.35 ± 2.03	1.95 ± 1.28[c]	91.8 ± 32.5
DN III ($n = 3$)	49.0 ± 7.9	10.7 ± 9.0	1.73 ± 0.31[d,e]	5.23 ± 0.35[b]	136.7 ± 35.5	10.40 ± 3.75	6.46 ± 2.03[d]	41.3 ± 8.6[e,f]

[a] Data are expressed as mean $\pm$ SD.
[b] $P < 0.01$ *versus* DN I.
[c] $P < 0.05$ *versus* DN I.
[d] $P < 0.001$ *versus* DN I.
[e] $P < 0.001$ *versus* DN II.
[f] $P < 0.05$ *versus* DN II.

Immunohistochemistry

Renal biopsy specimens were sectioned at 4 μm and fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) on ice for 15 min. After washing with PBS, the sections were blocked with 4% skim milk for 60 min at room temperature and subsequently incubated with either anti-AGE (CML), anti-pentosidine, anti-acrolein-protein adduct, anti-MDA-lysine, or anti-HNE-protein adduct antibody at 4°C overnight. After washing with Tris-saline buffer containing 100 mM NaCl and 150 mM Tris-HCl, pH 7.5, the sections were dehydrated through graded ethanol, incubated in methanol with 0.3% H_2O_2 at room temperature for 20 min to block endogenous peroxidase, and washed three times with Tris-saline buffer with 0.02% Tween 20 (Wako Pure Chemical Industries, Osaka, Japan). The sections for anti-CML, anti-acrolein-protein adduct, or anti-MDA-lysine antibody were incubated with rabbit anti-mouse IgG conjugated with peroxidase (Dako, Glostrup, Denmark), and the sections for anti-pentosidine

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Table 3. Clinical parameters of patients with IgA-N[a]

Histologic Grade	Age (yr)	Serum Creatinine (mg/dl)	Total Protein (g/dl)	Urinary Protein (g/d)	Creatinine Clearance (ml/min)
IgA-N I ($n = 5$)	43.2 ± 4.7	0.86 ± 0.17	6.24 ± 0.74	0.47 ± 0.42	76.6 ± 6.8
IgA-N II ($n = 5$)	47.4 ± 7.4	1.10 ± 0.24[b]	6.58 ± 0.54	1.33 ± 0.91[c]	62.8 ± 9.0
IgA-N III ($n = 5$)	46.8 ± 10.5	2.50 ± 0.91[d]	6.92 ± 0.70	1.64 ± 0.29[e]	50.3 ± 19.5[c]

[a] Data are expressed as mean ± SD.
[b] $P < 0.001$ *versus* IgA-N I.
[c] $P < 0.05$ *versus* IgA-N I.
[d] $P < 0.005$ *versus* IgA-N II.
[e] $P < 0.01$ *versus* IgA-N I.

Table 4. Antibodies used in the present study[a]

Antibody	Epitope (Origin)
Anti-pentosidine rabbit IgG (10, 35)	Pentosidine (carbohydrates, ascorbate)
Anti-AGE mouse IgG (39, 40)	CML (carbohydrates, ascorbate, PUFA, L-serine)
Anti-MDA-lysine mouse IgG (37, 38)	MDA-lysine (PUFA)
Anti-HNE-protein adduct rabbit IgG (36)	HNE-protein adducts (PUFA)
Anti-acrolein-protein adduct mouse IgG (31)	Acrolein-protein adducts (PUFA, L-threonine)

[a] AGE, advanced glycation end product; CML, carboxymethyllysine; PUFA, polyunsaturated fatty acids; MDA, malondialdehyde; HNE, 4-hydroxynonenal.

or anti-HNE-protein adduct antibody were incubated with swine anti-rabbit IgG conjugated with peroxidase (Dako). After washings with Tris-saline buffer containing Tween 20, they were developed by reactions with 3,3'-diaminobenzidine solution containing 0.03% H_2O_2, followed by the counterstaining with hematoxylin. Immunostaining for intensity was independently evaluated by three observers and graded blindly as trace (±), weak (+), and strong (++). Competition experiments to confirm the specificity of immunostaining were performed with anti-CML, anti-pentosidine, anti-HNE-protein adduct, anti-MDA-lysine, or anti-acrolein-protein adduct antibody, which had been preincubated with an excess of CML-bovine serum albumin (BSA), pentosidine, HNE-BSA, MDA-BSA, or acrolein-BSA, respectively, for 4 h at 37°C, as described previously (10,35). Nonimmune rabbit or mouse IgG was used as a negative control.

Results

Results of immunohistochemical studies in renal tissues from control subjects and from patients with DN or IgA-N are

summarized in Table 5. We focused on the immunostaining within the glomerulus.

The antibodies for CML (Figure 2A, left panel), pentosidine (Figure 2A, right panel), MDA-lysine (Figure 3A, left panel), HNE-protein adduct (Figure 3A, right panel), and acrolein-protein adduct (Figure 4A) did not stain the glomeruli in normal renal tissues from all five control subjects.

DN is characterized by an expanded mesangial matrix, a thickened capillary wall, and nodular deposits. In renal tissues of DN patients, glomeruli were stained positive for CML (Figure 2B, left panels), pentosidine (Figure 2B, right panels), MDA-lysine (Figure 3B, left panels), HNE-protein adduct (Figure 3B, right panels), and acrolein-protein adduct (Figure 4B) in all of the DN patients. Immunostaining was mainly observed in the expanded mesangial area and capillary walls. Nodular lesions within the glomeruli were also strongly stained

Table 5. Results of immunostaining for glycoxidation products, lipoxidation products, and acrolein-protein adduct in healthy subjects and patients with DN or IgA-N[a]

Group	Grade	CML	Pentosidine	MDA-Lysine	HNE-Protein Adduct	Acrolein-Protein Adduct
Healthy subjects		±	±	±	±	±
DN	I	±	+	+	±~+	±~+
	II	+	+~++	+~++	+	+
	III	+~++	++	++	+~++	++
IgA	I	±	±	±	±	±
	II	±	±	±~+	±~+	±
	III	±	±	+~++	+~++	±

[a] Abbreviations as in Table 4.
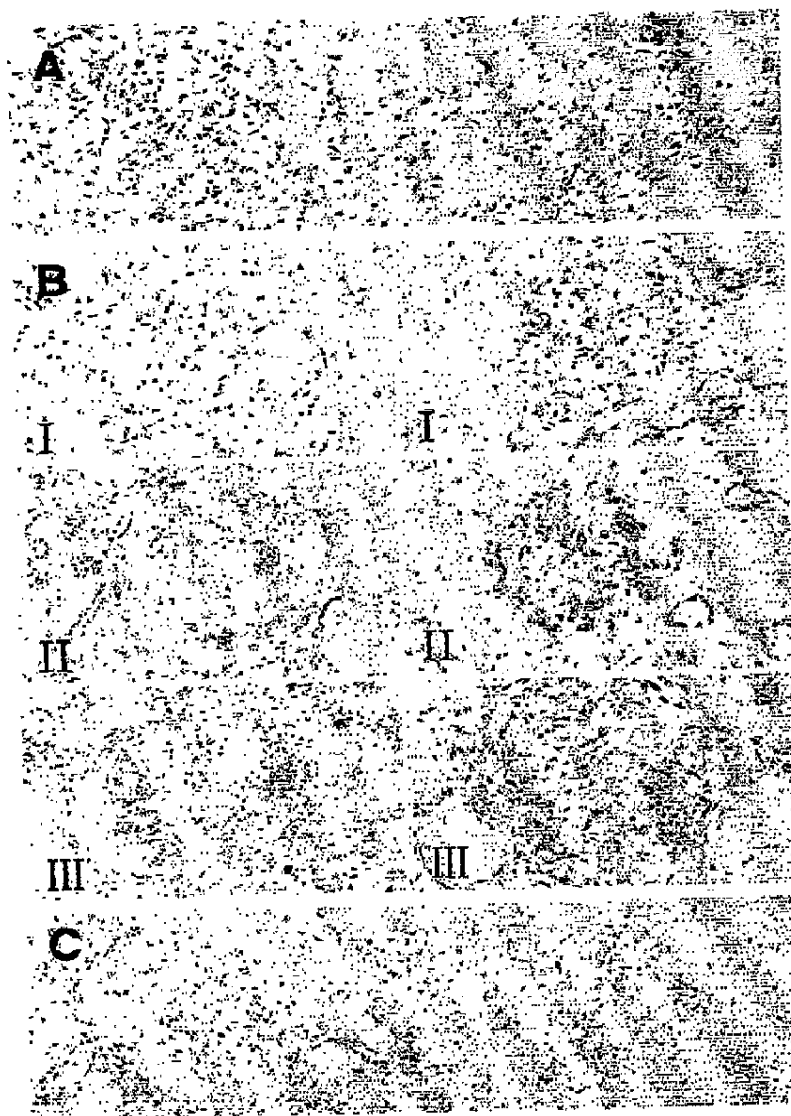±, trace; +, weak; ++, strong.

Figure 2. Immunohistochemical detection of glycoxidation products, carboxymethyllysine (CML) (left panel), and pentosidine (right), in normal renal tissues (A) or DN (B and C). The CML and pentosidine antibodies did not stain the glomeruli in control subjects (A). By contrast, in the glomeruli of DN (B), both CML and pentosidine were stained positive in the expanded mesangial area (top and middle panels) and in nodular lesions (bottom). The specificity of immunostaining was confirmed by competition experiments with either anti-CML or anti-pentosidine antibody preincubated with an excess of CML-bovine serum albumin (BSA) or pentosidine, respectively. Glomeruli did not stain with either anti-CML or anti-pentosidine antibody in the presence of competitors (C). Magnification, ×200.

positive for all five biomarkers (Figures 2, 3, and 4B, lower panels). The intensity of their immunostainings appeared to increase with the histologic and clinical severities of DN (Figure 1A and Table 2). Immunostaining was specific as demonstrated by its complete inhibition in the presence of an excess of competitors (Figures 2 through 4C). No immunoreaction was observed with nonimmune mouse or rabbit IgG (data not shown).

In renal tissues from patients with IgA-N, glomeruli reacted with both anti-MDA-lysine (Figure 5, left panels) and anti-HNE-protein adduct antibodies (Figure 5, right panels). Immunostaining was mainly observed in the expanded mesangial area and vascular poles. The intensity of the immunostaining for MDA-lysine and HNE-protein adduct appeared to increase with the histologic and clinical severities of IgA-N (Figure 1B and Table 3). By contrast, CML (Figure 6, left panel), pentosidine (Figure 6, middle panel),
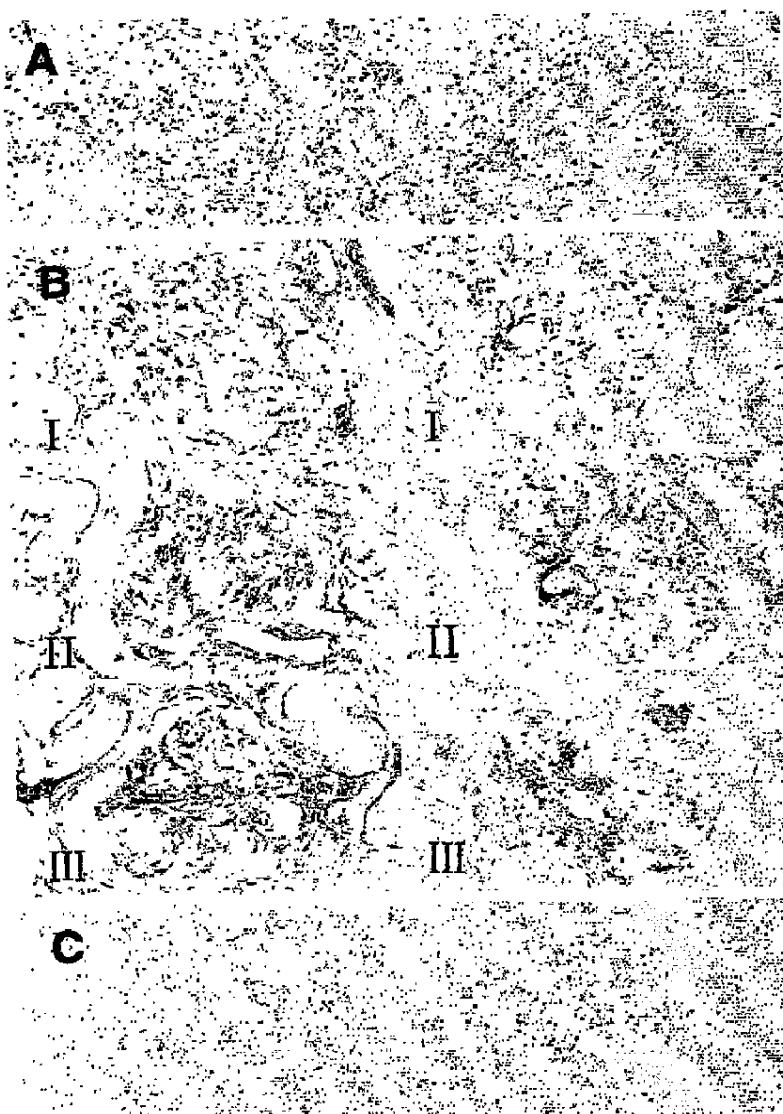
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Figure 3. Immunohistochemical detection of lipoxidation products, malondialdehyde-lysine (MDA-lysine) (left panel), and 4-hydroxynonenal (HNE)-protein adduct (right), in normal renal tissues (A) or DN (B and C). The MDA-lysine and HNE-protein adduct antibodies did not stain the glomeruli in control subjects (A). In the glomeruli of DN (B), however, both MDA-lysine and HNE-protein adduct were stained positive in the expanded mesangial area (top and middle panels) and in nodular lesions (bottom). Glomeruli did not stain with either anti-MDA-lysine or anti-HNE-protein adduct antibody in the presence of an excess of MDA-BSA or HNE-BSA, respectively (C). Magnification, ×200.

and acrolein-protein adduct (Figure 6, right panel) immunoreactivities were only faint in glomeruli.

Discussion

Glycoxidation products are combined products of glycation and oxidation of proteins, whereas lipoxidation products and acrolein-protein adduct are oxidation-specific lipid- and/or amino acid-protein adducts. Glycoxidation products, lipoxidation products, and acrolein-protein adduct were identified im-

munohistochemically in the characteristic DN lesions, i.e., in the expanded mesangial matrix and in nodular lesions. This pattern is specific for DN: Glycoxidation product, lipoxidation product, and acrolein-protein adduct immunoreactivities were only faint in normal glomeruli from control subjects, and glycoxidation product and acrolein-protein adduct immunoreactivities were faint in glomeruli from patients with IgA-N.

The antibodies used in the present study recognize distinct structures and do not cross-react with the other structures.

Figure 4. Immunohistochemical detection of acrolein-protein adduct in normal renal tissues (A) or DN (B and C). The acrolein-protein adduct antibody did not stain the glomeruli in control subjects (A). By contrast, acrolein-protein adduct was stained positive in the expanded mesangial area (top and middle panels) and in nodular lesions (bottom) of DN (B). Glomeruli were not stained with anti-acrolein-protein adduct antibody in the presence of an excess of acrolein-BSA (C). Magnification, ×200.

Therefore, the distributions of all of the protein adducts formed by carbonyl amine chemistry in DN are independent measures for an increased oxidative stress and carbonyl modification of proteins. Under conditions of enhanced oxidative stress in diabetic glomerular lesions, carbohydrates, lipids, and amino acids might be autoxized by reactive oxygen species and converted to reactive carbonyl compounds, which, in turn, react with proteins, leading to formation of glycoxidation products, lipoxidation products, and acrolein-protein adduct on proteins. The increased protein modification with reactive carbonyl compounds derived from autoxidation products of carbohydrates, lipids, and amino acids would be described more appropriately as "carbonyl stress," rather than advanced glycation or oxidative stress alone.

In addition to the nonenzymatic mechanism of the formation of reactive carbonyl compounds, the segmental nature of the immunostaining, e.g., CML and acrolein-protein adduct, may have to do with an enzymatic mechanism of the formation of reactive carbonyl compounds based on glycolaldehyde and 2-hydroxypropanal formation upon action of myeloperoxidase-mediated Strecker degradation of serine and threonine (32). This additional mechanism of CML and acrolein-protein adduct formation further supports the concept of a localized oxidative stress.

Proximal renal tubular cells were stained positive for pentosidine and MDA-lysine in normal renal tissues and in renal tissues from both DN and IgA-N. As described previously (10), detection of these adducts within proximal tubular cells might reflect the tubular reabsorption of free-form adducts (42,43). We indeed demonstrated that circulating free-form pentosidine is continuously filtered through the glomerulus and reabsorbed by the proximal renal tubule in rats administered intravenously with free-form pentosidine (42–44).

In renal tissues from IgA-N, MDA-lysine and HNE-protein adduct were stained positive in the expanded mesangial area, whereas CML, pentosidine, and acrolein-protein adduct immunoreactivities were only faint in glomeruli. *In vitro* studies demonstrated that the yields of CML, pentosidine, MDA-lysine, and HNE-protein adduct on BSA after incubation for 4 wk at 37°C under atmospheric oxygen (taken as an oxidative stress), with either 100 mM glucose (for CML and pentosidine) or 20 mM arachidonate (for MDA and HNE) in 0.1 M phosphate buffer, pH 7.4, were ~5 nmol, ~200 pmol, ~10 nmol, and ~2 μmol expressed by mg of albumin, respectively (45). This suggests that the level of reactive oxygen species (oxidative stress) necessary for generation of reactive carbonyl compounds from carbohydrates is much higher than that for reactive carbonyl compounds from lipids. Therefore, one explanation for the absence of CML, pentosidine, and acrolein-protein adduct might be that the level of oxidative stress in glomeruli of IgA-N might be much lower than that in DN and does not reach the threshold that converts carbohydrates or amino acids to reactive carbonyl compounds, or not reach the levels detectable by the present immunohistochemical methods. If the latter is the case, the glomerular lesions in IgA-N also appear to be a state of increased carbonyl stress. Alternatively, some unknown mechanisms may exist in IgA-N to detoxify the reactive carbonyl compounds derived from hydrophilic carbohydrates and amino acids, but not from hydrophobic lipids. More studies will be required to address the issue of the presence of carbonyl stress in IgA-N.

The pathologic significance of carbonyl stress in diabetic glomerular lesions is another issue of particular interest. Two
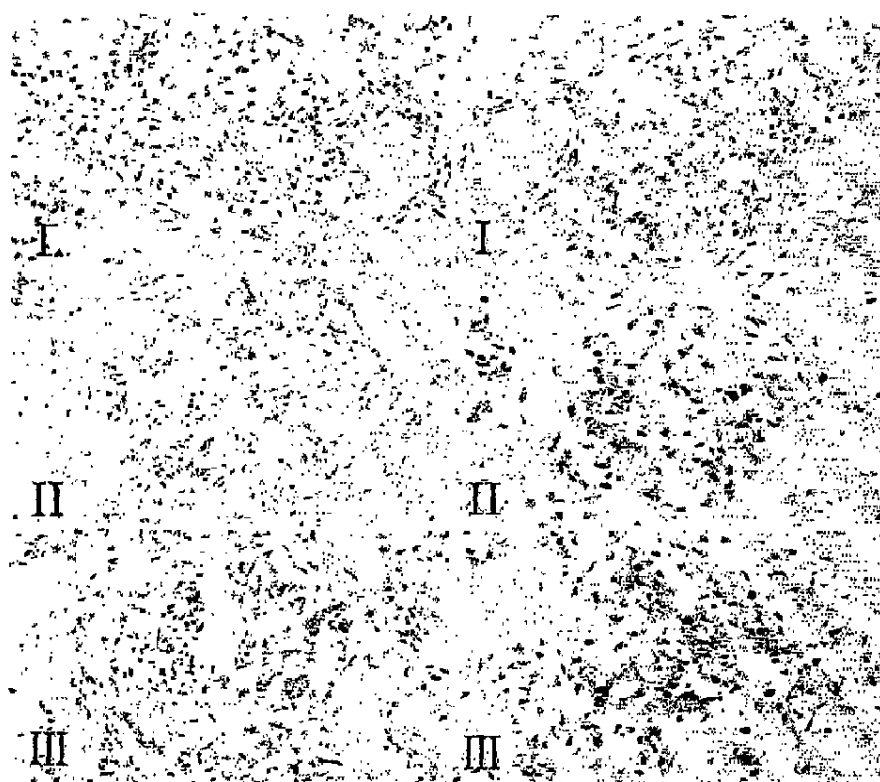
Figure 5. Immunohistochemical detection of lipoxidation products, MDA-lysine (left panel), and HNE-protein adduct (right) in IgA-N. Both MDA-lysine and HNE-protein adduct were stained positive in the expanded mesangial area. Magnification, ×200.

possible mechanisms should be considered regarding the role of carbonyl stress in glomerular tissue damage. First, the final protein adducts resulting from carbonyl stress, such as AGE-modified proteins, stimulate a variety of cellular responses including glomerular mesangial cells (16,17). AGE indeed stimulate fibronectin (16) and type IV collagen (17) synthesis in cultured human or mouse mesangial cells. Second, carbonyl stress not only induces an inflammatory response, but also may have direct biologic effects on parenchymal cells. Reactive carbonyl compounds and residual carbonyl groups on AGE-modified proteins react covalently with matrix tissue proteins and alter the structure and function of matrix tissue proteins. Or, they react with cell surface proteins and stimulate cellular responses by cross-linking of cell surface proteins (46,47). A number of intracellular proteins are phosphorylated on tyrosine residues in murine thymocytes exposed to reactive carbonyl compounds, such as MDA, glyoxal or HNE (48). These data indicate that the interaction of reactive carbonyl compounds, either free or protein-bound, with cell surface membrane proteins can induce intracellular responses. In this study, we have shown evidence for an increased carbonyl stress in DN using immunohistochemical staining. Additional studies that examine directly evidence for the relationship between carbonyl stress and pathogenesis of DN are therefore warranted.

The carbonyl stress theory in diabetic glomerular lesions would provide a useful insight to the treatment of diabetic glomerular tissue damage. If the generation of reactive carbonyl compounds is not a mere result of oxidative stress but is an active contributor to the pathogenesis, an inhibition of carbonyl group reaction with proteins might prevent tissue damage. We demonstrated that OPB-9195 (±2-isopropyli-denehydrazono-4-oxo-thiazolidin-5-ylacetanilide), a drug that belongs to a group of thiazolidine derivatives (49), as well as aminoguanidine (50), inhibit the formation of Schiff base and eventually all of the protein adducts formed with reactive carbonyl compounds, including glycoxidation products and lipoxidation products (45,51,52). Both contain a hydrazine nitrogen atom that reacts with carbonyl groups, directly or *via* the base upon hydrolysis, to form hydrazone. These compounds are therefore thought to entrap reactive carbonyl compounds, either free or attached to proteins. Indeed, aminoguanidine given to streptozotocin-induced diabetic mice (53) or OPB-9195 given to Otsuka-Long-Evans-Tokushima-Fatty (OLETF) rats (49), a model of non-insulin-dependent diabetes mellitus, reduces urinary albumin excretion as well as mesangial expansion in the glomeruli. Undoubtedly, more studies will be required to test the usefulness of reactive carbonyl compound entrapping in the treatment of diabetic glomerular tissue damage.
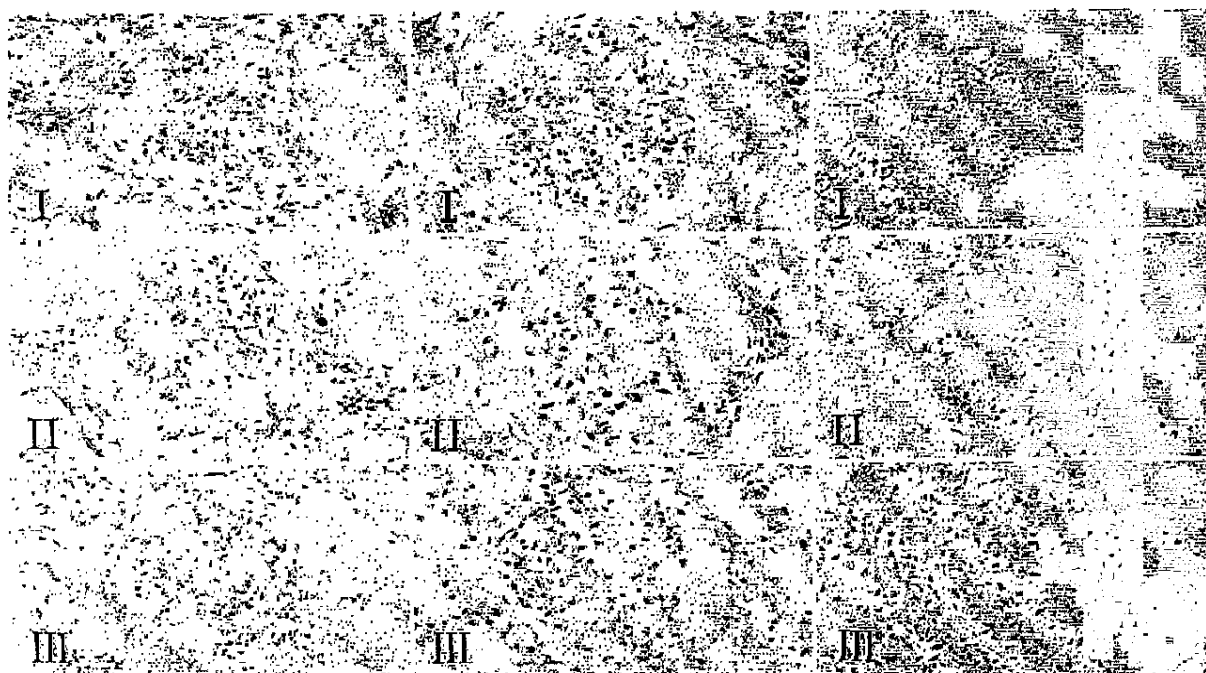
Figure 6. Immunohistochemical detection of glycoxidation products, CML (left panel) and pentosidine (middle), and acrolein-protein adduct (right) in IgA-N. The CML, pentosidine, and acrolein-protein adduct antibodies did not stain the glomeruli of IgA-N. Magnification, ×200.

Acknowledgments

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